VERZONDEN W FFF V

REPORT

PRIMARY SKIN IRRITATION/CORROSION STUDY WITH

IN THE RABBIT
(4-HOUR SEMI-OCCLUSIVE APPLICATION)

NOTOX Project 338682 NOTOX Substance 111834/B

- Page 1 of 12 -

CONFIDENTIALITY STATEMENT

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STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with the most recent edition of:

The OECD Principles of Good Laboratory Practice which are essentially in conformity with:

United States Environmental Protection Agency (FIFRA). Title 40 Code of Federal Regulations Part 160.

United States Environmental Protection Agency (TSCA). Title 40 Code of Federal Regulations Part 792.

United States Food and Drug Administration. Title 21 Code of Federal Regulations Part 58.

Japanese Ministry of Agriculture, Forestry and Fisheries. 59 NohSan, Notifications No. 3850.

Japanese Ministry of Economy, Trade and Industry. Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85.

Japanese Ministry of Health, Labor and Welfare. Ordinance No.21.





QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands

This report was audited by the NOTOX Quality Assurance Unit to ensure that the methods and results accurately reflect the raw data.

The dates of Quality Assurance inspections and audits are given below.

During the on-site inspections procedures applicable to this type of study were inspected.

DATES OF QAU INSPECTIONS/ AUDITS	REPORTING DATES						
on-site inspection(s)							
08-16 October 2001 (process)	25 October 2001						
protocol inspection(s)							
08 November 2001	08 November 2001						
report audit(s)							
16 January 2002 (study)	16 January 2002						
Head of Quality Assurance:							

SUMMARY
Primary skin irritation/corrosion study with occlusive application).
The study was carried out based on the guidelines described in: EC Commission Directive 92/69/EEC, B.4, "Acute Toxicity - Skin irritation" and OECD No.404, "Acute Dermal Irritation/Corrosion".
Three rabbits were exposed to 0.5 ml of applied onto clipped skin for 4 hours using a semi-occlusive dressing. Observations were made 1, 24, 48 and 72 hours and 7 and 14 days after exposure.
Four hours exposure to resulted in severe erythema and moderate to severe oedema in the treated skin-areas of the three rabbits.
After removal of the bandage, white discolouration of the treated skin site was seen among all animals, which was considered to be a sign of (beginning) necrosis. Grey discolouration of the skin (a sign of necrosis) and eschar formation was noted at 1, 24, 48 and/or 72 hours after exposure among all animals. In addition, scabs were noted among the animals during the observation period. At 14 days after exposure, a bald, noticeably white skin, and scaliness of the treated skin site was noted among all animals.
Due to eschar formation and scabs, oedema could not be scored at several time points during the observation period among all animals.
Since sufficient infomation was obtained and no reversibility of the effects was to be expected, the study was terminated after the 14 days observation.
As evidence of full thickness destruction of the skin (scar formation; evidenced by the notably white skin) was obtained during the observation period, it was concluded that corrosion of the skin had occurred by dermal application of
Based on the results and according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC)

PREFACE

Sponsor

Study Monitor

SHERA, Regulatory Affairs

Testing Facility

NOTOX B.V.

Hambakenwetering 7

5231 DD 's-Hertogenbosch

The Netherlands

Study Director

Study Plan

Start: 04 December 2001

End: 18 December 2001

TEST SUBSTANCE

The sponsor is responsible for all test substance data unless determined by NOTOX.

Identification Chemical name

CAS RN

Clear colourless liquid

Description Batch

1510-14

Purity

Density

See Certificate of Analysis In refrigerator in the dark

Test substance storage

Stable

Stability under storage conditions Expiry date

01 January 2003 Approx. 1160 kg.m⁻³

TEST SUBSTANCE PREPARATION

The test substance was applied undiluted as delivered by the sponsor.

PURPOSE AND RATIONALE

The purpose of this primary skin irritation study was to assess the possible irritation or corrosion potential of a single dose of the test substance when administered to the intact skin of rabbits. This study should provide a rational basis for risk assessment in man.

The absence of skin pigmentation in the albino rabbit facilitates the evaluation of induced skin reactions. The dermal route was selected because the test substance may accidentally come into contact with the skin during manufacture, handling and/or use.

GUIDELINES

As required by the Dutch Act on Animal Experimentation, the study protocol was reviewed and agreed by the Article 14-functionary and the Ethical Committee of NOTOX (DEC NOTOX 97-03-09) as required by the Dutch Act on Animal Experimentation (February 1997). The study procedures described in this report were based on the following guidelines:

European Community (EC), Council Directive 67/548/EEC, Annex V, Part B, Methods for the Determination of Toxicity, as last amended by Commission Directive 92/69/EEC, B.4: "Acute Toxicity - Skin Irritation". Official Journal of the European Communities No. L 383, 1992

Organisation for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals, Section 4, Health Effects, No.404: "Acute Dermal Irritation / Corrosion", Paris Cedex, 1992.

ARCHIVING

NOTOX B.V. will archive for at least 10 years raw data, protocol, report and test substance reference sample. No data will be withdrawn without the sponsor's written consent.

TEST SYSTEM

Species Albino Rabbit, New Zealand White, (SPF-Quality)

Recognised by international guidelines as the recommended test

system (e.g. EC, OECD)

Source: Charles River Deutschland, Kisslegg, Germany.

Number of animals

3 Animals of one sex.

Age and body weight Animals used within the study were at least 6 weeks old and body

weights were between 1.0 and 3.5 kg.

Identification Earmark.

ANIMAL HUSBANDRY

Conditions

A controlled environment was maintained in the room with optimal conditions considered as being approximately 15 air changes per hour, a temperature of 21±3°C, a relative humidity of 30-70% and 12 hours artificial fluorescent light and 12 hours dark per day.

Deviations from the maximum level for relative humidity (with a maximum of 7%) occurred which might have been caused by cleaning procedures in the room. Based on laboratory historical data these deviations were considered not to have affected the study integrity.

Accommodation

Individually in labelled cages with perforated floors (Scanbur, Denmark, dimensions 53.5x63x38.5 cm). Acclimatisation period was at least 5 days before start of treatment under laboratory conditions.

Diet

Standard laboratory rabbit diet (Charles River Breeding and Maintenance Diet for Rabbits, Altromin, Germany) approx. 100 g. per day. Certificates of analysis were examined and retained in the NOTOX archives. In addition, hay (BMI, Helmond, the Netherlands) was provided twice a week.

Water

Free access to tap-water. Certificates of quarterly analysis were examined and retained in the NOTOX archives.

TREATMENT

Approximately 24 hours before treatment, the dorsal fur was clipped with electric clippers, exposing an area of approximately 150 square centimeters (10x15 cm²). Whenever considered necessary the treated skin areas were re-clipped at least 3 hours before the observations, to facilitate scoring.

A health inspection was performed prior to the commencement of treatment, to ensure that the animals were in a good state of health. Special attention was paid to the skin to be treated, which was intact and free from abnormalities.

Each animal was treated by dermal application of 0.5 ml of the test substance. The test substance was applied to the skin of one flank, using a metalline patch* of 2x3 cm. The patch was mounted on Micropore tape', which was wrapped around the abdomen and secured with Coban elastic bandage'.

Four hours after the application, the dressing was removed and the skin cleaned of residual test substance using water.

- *. Supplier: Lohmann GmbH, Neuwied, Germany.
- *. Supplier: 3M, St. Paul, Minnesota, U.S.A.

OBSERVATIONS

Mortality/Viability

Twice daily.

At least once daily.

Body Weight

Day of treatment (prior to application).

Irritation

The skin reactions were assessed at approximately 1, 24, 48 and 72 hours and 7 and 14 days after the removal of the dressings and test substance. The irritation scores and a description of all other (local) effects were recorded. Adjacent areas of the untreated skin

The irritation was assessed according to the following numerical scoring system. At each observation, the highest scores given was recorded:

of each animal served as controls.

Erythema and eschar formation:

No erythema	. 0
Very slight erythema (barely perceptible)	
Well-defined erythema	
Moderate to severe erythema	
Severe erythema (beet redness) *	
*	

[.] Where signs of necrosis or corrosion (injuries in depth) prevent erythema scoring, the maximum grade for erythema (= 4) is given.

Oedema formation:
No oedema 0
Very slight oedema (barely perceptible) 1
Slight oedema (edges of area well-defined by definite raising)
Moderate oedema (raised approximately 1 millimeter)
Severe oedema (raised more than 1 millimeter and extending beyond the area of exposure)4
HISTOPATHOLOGY
No histopathology was performed.
INTERPRETATION
The results were evaluated according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC).
RESULTS
Four hours exposure to 0.5 ml of 8 resulted in severe erythema and moderate to severe oedema in the treated skin-areas of the three rabbits.
After removal of the bandage, white discolouration of the treated skin site was seen among all animals, which was considered to be a sign of (beginning) necrosis. Grey discolouration of the skin (a sign of necrosis) and eschar formation was noted at 1, 24, 48 and/or 72 hours after exposure among all animals. In addition, scabs were noted among the animals during the observation period. At 14 days after exposure, a bald, noticeably white skin, and scaliness of the treated skin site was noted among all animals.
Due to eschar formation and scabs, oedema could not be scored at several time points during the observation period among all animals (see table 1).
Since sufficient infomation was obtained and no reversibility of the effects was to be expected, the study was terminated after the 14 days observation.
Colouration
No staining of the treated skin by the test substance was observed.
Toxicity / Mortality No symptoms of systemic toxicity were observed in the animals during the test period and no mortality occurred.
CONCLUSION
As evidence of full thickness destruction of the skin (scar formation; evidenced by the notably white skin) was obtained during the observation period, it was concluded that corrosion of the skin had occurred by dermal application of
Based on the results and according to the EC criteria for classification and labelling requirements for dangerous substances and preparations (Guidelines in Commission Directive 93/21/EEC), should be labelled as: causes burns (R 34).

TABLE 1: INDIVIDUAL SKIN IRRITATION SCORES

Following exposure, white discolouration of the treated skin-area was noted after removal of the test substance.

Animal #	4			5			6		
Time after exposure	Erythema	Oedema	comments	Erythema	Oedema	comments	Erythema	Oedema	comments
1 hour	4	3	k	4	4	k	4	4	k
24 hours	4	2	k	4	-	kd	4	2	k
48 hours	4	2	k	4	-	kde	4	-	k d
72 hours	4	-	k d	4	-	d e	4	-	d
7 days	4	-	е	4	-	е	4	-	е
14 days	1	2	hjl	4	_	ehjl	1	2	hjl

Comments:

- No scoring possible due to d and/or e. -. d.
- Eschar formation.
- e. h.
- Scabs. Bald skin.
- j k. l.
- Bald, notably white skin.
 Grey discolouration, a sign of necrosis.
 Scaliness.

Animal specifications (at start of study):

Animal no	Sex	Age (weeks)	Body weight (grams)		
4	♂	7-9	1635		
5	♂	7-9	1585		
6	8	7-9	1695		



Certificate of Analysis Polymer Chemicals

TNA-2001007 page 1 of 2

ICS-331

Product name Chemical name: Batch number: 1510-14

Test results:

Method	Analysis of		Unit	Result *1
Jo/72.11,				
	ation	·		
J20010792	D	e	% m/m	67.0 (± 1.0)
J20010792	IU		% m/m	2.0 (± 0.3)
Amp/88.9	Water		% m/m	2.6 (± 0.3)
J20010792	Unidentified impurities		% m/m	0.5 (± 0.2)

^{*1} bracketed values are estimated 95% confidence intervals

File code

: TNA-2001007

Analytical documentation

: 20010792

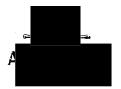
Authorized by

Name

Section Head, Analytical Research Department

October 25, 2001 Date

Signature:



Certificate of Analysis Polymer Chemicals

TNA-2001007 page 2 of 2

